



Low-energy formation of edible nanoemulsions by spontaneous emulsification: Factors influencing particle size



Jennifer Komaiko^a, David Julian McClements^{a,b,*}

^a Department of Food Science, 102 Holdsworth Way, University of Massachusetts, Amherst, MA 01003, USA

^b Department of Biochemistry, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia

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ABSTRACT

Nanoemulsions are used as delivery systems in food, pharmaceutical, and personal care applications for a variety of lipophilic active components, e.g., antimicrobials, flavors, colors, preservatives, vitamins, nutraceuticals, and drugs. In this study, we examined the effect of system composition and preparation conditions on the production of edible nanoemulsions using spontaneous emulsification (SE). SE is a low-energy method that simply involves addition of an organic phase (oil + surfactant) into an aqueous phase. The influence of surfactant-to-oil ratio (SOR), surfactant type, surfactant location, and oil type were tested. The droplet size produced decreased with increasing SOR, and was smallest when the non-ionic surfactant Tween 80 was used. Smaller droplets were formed when the surfactant was initially dispersed in the oil phase rather than the aqueous phase. Ten food-grade oils were tested and we found that droplet size followed the order: medium chain triglycerides < flavor oils < long chain triglycerides. No correlation was found between droplet size and the physicochemical characteristics of the oil phase (refractive index, density, interfacial tension, and viscosity). Results obtained by spontaneous emulsification were correlated to those obtained by emulsion phase inversion on similar systems suggesting a common underlying physicochemical mechanism.

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1. Introduction

Nanoemulsions have been defined as emulsions with mean droplet diameters < 200 nanometers, *i.e.*, radius < 100 nm (Solans et al., 2005). They are of particular interest in the food, supplement, and pharmaceutical industries because their small particle size can lead to delivery systems with high optical clarity, good kinetic stability, and high oral bioavailability (Bouchemal et al., 2004; McClements, 2011; Sonneville-Aubrun et al., 2004). Nanoemulsions can be fabricated using either high-energy or low-energy approaches, which can be distinguished based on the physicochemical mechanisms involved. High-energy approaches rely on specialized equipment (“homogenizers”) to disrupt and intermingle the oil and water phases, thus forming small droplets (Abbas et al., 2013). In contrast, low-energy approaches require no special equipment and utilize the properties of the surfactant, oil, and water system to spontaneously form nanoemulsions based on simply mixing procedures or by changing system conditions such as temperature (Schalbart and Kawaji, 2013; Solans and Sole, 2012).

* Corresponding author at: Department of Food Science, 102 Holdsworth Way, University of Massachusetts, Amherst, MA 01003, USA. Tel.: +1 (413) 545 1019; fax: +1 (413) 545 1262.

E-mail address: mcclements@foodsci.umass.edu (D.J. McClements).

Low energy methods are of interest due to their low cost and ease of implementation (Komaiko and McClements, 2014), which has led to an increasing amount of research into the development and application of various low energy approaches (Gulotta et al., 2014; Komaiko and McClements, 2014; Mayer et al., 2013a; Ostertag et al., 2012; Saberi et al., 2013). Low energy approaches can be broadly divided into isothermal and thermal methods. Isothermal methods, such as spontaneous emulsification (SE) and emulsion phase inversion (EPI), do not require any temperature changes to form nanoemulsions (Komaiko and McClements, 2014). Instead, they are based on the spontaneous formation of ultrafine droplets at the boundary between an organic and aqueous phase of specific composition when they are brought into contact. The spontaneous formation of nanoemulsions by isothermal methods can be achieved using various methods: (1) simply mixing oil, water, and water-miscible solvent together (Ganachaud and Katz, 2005); (2) contact of an oil, hydrophobic surfactant, and water-miscible solvent mixture with an aqueous phase (Bouchemal et al., 2004); and (3) addition of an oil and hydrophilic surfactant mixture into an aqueous phase (Anton and Vandamme, 2009). In food grade systems, process (3) is of particular interest due to the fact that a solvent is not necessary. The proposed mechanism for spontaneous emulsification by this method is the rapid

diffusion of hydrophilic surfactant from the organic phase to the aqueous phase when they come into contact (Anton and Vandamme, 2009) (Fig. 1).

Other researchers have examined the factors affecting the spontaneous emulsification process using non-food grade components and solvents (Bouchemal et al., 2004). Our laboratory has previously examined the factors affecting the size of oil droplets produced using the EPI method with food grade components (Ostertag et al., 2012). The EPI method involves titrating an aqueous phase into an organic phase containing oil and hydrophilic surfactant. This process initially leads to the formation of a water-in-oil emulsion (W/O), then an oil-in-water-in-oil emulsion (O/W/O) and then an oil-in-water emulsion (O/W). The goal of the current research was to determine the factors affecting the size of the droplets produced using the SE method with food grade components. The SE method is also an isothermal low-energy method, but it involves titrating an organic phase containing oil and hydrophilic surfactant into an aqueous phase. Previous research has suggested that there are some similarities and differences between these two different isothermal low-energy methods (Mayer et al., 2013b; Ostertag et al., 2012; Saberi et al., 2013). One of the aims of this study was to compare the size of the droplets produced using the SE and EPI methods on similar surfactant–oil–water systems. The experiments were therefore intentionally designed so that a direct comparison could be drawn between the two methods.

2. Materials and methods

2.1. Materials

Ten different oil phases were used to prepare the emulsions (Table 1). Medium chain triglycerides (MCT, Miglyol 812N, Warner Graham Company, Cockeysville, MD), orange oil (10×, Item No. 49024, The Chemistry Store, Cayce, SC), and Mineral Oil (Sigma-Aldrich, St. Louis, MO) were bought from chemical suppliers. Lemon oil (3×, Citrus & Allied Essences, Lake Success, NY) and fish oil (Ropufa 30 n-3 food oil, DSM Nutritional Products Ltd., Basel, Switzerland) were kindly donated. Grapeseed oil, toasted sesame oil, canola oil, peanut oil, and extra virgin olive oil were bought from a local grocery store. A variety of non-ionic surfactants were used (Table 2) including Span® 20, Tween® 20, 40, 60, 80 and 85 (Sigma-Aldrich, St. Louis, MO). The aqueous phase for all emulsions was a sodium phosphate buffer solution (5 mM; pH 7.0).

Distilled and deionized water (Milli-Q®) was used to prepare all solutions and emulsions.

2.2. Methods

2.2.1. Emulsion preparation

Emulsions were prepared by spontaneous emulsification which involves titrating an organic phase into an aqueous phase. In most experiments, the organic phase consisted of oil and surfactant. The experiments were performed in a 50 ml beaker at ambient temperature ($\approx 20^\circ\text{C}$). The experiments were designed so that the final emulsion always had a total mass of 25 g including 2.5 g of oil (i.e., 10 wt% oil). Initially, an organic phase was prepared by adding the surfactant and oil to the beaker and then mixing using a magnetic stirrer (500 rpm) for a minimum of 30 min. The thoroughly mixed organic phase was then added to a stirring aqueous phase (750 rpm) over 5 min using a programmable automated pipette (Rainin SE4, Mettler Toledo, Oakland, CA). An additional 5 min was allowed for mixing to bring the total preparation time to 10 min. Previous studies with a model system showed that there was no added benefit to increasing the processing time further (Komaiko and McClements, 2014).

2.2.2. Variables tested

Four main variables were tested: surfactant-to-oil ratio, type of surfactant, surfactant location, and oil type.

2.2.2.1. Influence of surfactant-to-oil ratio. The influence of surfactant concentration was investigated by varying the surfactant-to-oil ratio (SOR). The total oil content in the final systems was held constant at 10%, while the SOR was varied by altering the amounts of surfactant and water in the final system:

$$\text{SOR} = m_s/m_o \quad (1)$$

$$m_w = 100 - m_o - m_s \quad (2)$$

Here, m_s , m_o and m_w are the mass percentages of surfactant, oil and water, respectively. SORs tested included 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, and 2. All these tests were carried out using medium chain triglycerides (MCT) as the oil and Tween 80 as the surfactant.

2.2.2.2. Influence of surfactant type. A number of nonionic surfactants were used to stabilize the emulsions (Table 2) including Tween®20, 40, 60, 80 and 85 as well as Span®20. SOR was held constant at 2 and MCT was used as the oil phase for all experiments.

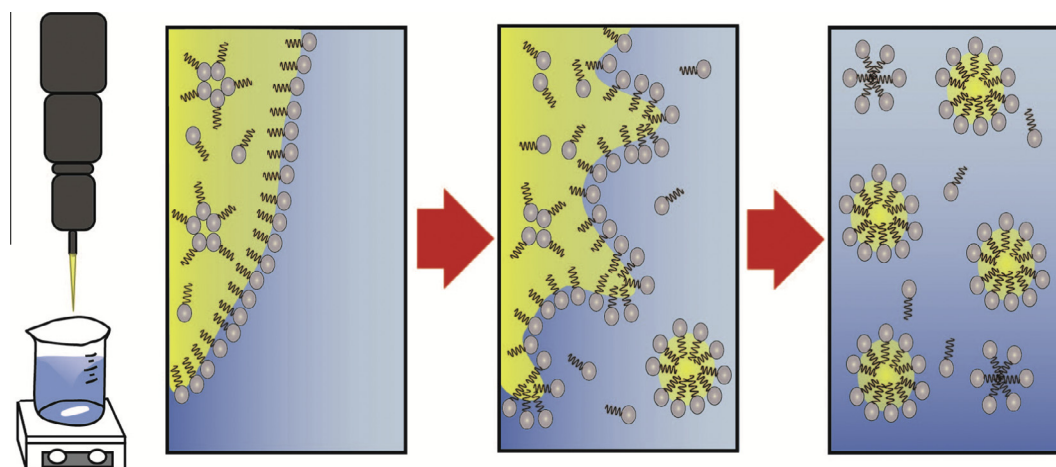


Fig. 1. Schematic representation of the spontaneous emulsification method. An organic phase (oil + surfactant) was added in intervals from a pipette into a constantly stirring aqueous phase (buffer solution) in a glass beaker using a magnetic stirrer. A proposed molecular view is included.

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